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(54) Title: A STABILIZED PHARMACEUTICAL FORMULATION COMPRISING A GROWTH HORMONE PRE-TREATED WITH ZINC AND OPTIONALLY LYSINE OR CALCIUM IONS

(57) Abstract

A pharmaceutical formulation comprising a growth hormone pre-treated with zinc and optionally lysine or calcium ions shows a very high stability against deamidation, oxidation and cleavage of peptide bonds. The stability of the product allows for the storing and shipment thereof in a lyophilized state or in the form of a dissolved or re-dissolved formulation at ambient temperature.

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TITLE

A STABILIZED PHARMACEUTICAL FORMULATION COMPRISING A GROWTH HORMONE PRE-TREATED WITH ZINC AND OPTIONALLY LYSINE OR CALCIUM IONS

FIELD OF THE INVENTION

The present invention relates to a stabilized pharmaceutical formulation comprising growth hormone, to a method of making such formulation, to the use of zinc for stabilizing a formulation of growth hormone, and to a method for treating a disorder affectable by growth hormone.

BACKGROUND OF THE INVENTION

The growth hormones from man and from the common domestic animals are proteins of approximately 191 amino acids, synthesized and secreted from the anterior lope of the pituitary gland. Human growth hormone consists of 191 amino acids.

Growth hormone is a key hormone involved in the regulation of not only somatic growth, but also in the regulation of metabolism of proteins, carbohydrates and lipids. The major effect of growth hormone is to promote growth.

The organ systems affected by growth hormone include the skeleton, connective tissue, muscles, and viscera such as liver, intestine, and kidneys.

Until the development of the recombinant technology and the cloning of the growth hormone gene now giving rise to production of e.g. human growth hormone (hGH) and Met-hGH in industrial scale, human growth hormone could only be obtained by extraction from the pituitary glands of human cadavers. The very limited supplies of growth hormone restricted the use thereof to longitudinal growth promotion in childhood and puberty for treatment of dwarfism, even

though it has been proposed for inter alia treatment of short stature (due to growth hormone deficiency, normal short stature and Turner syndrome), growth hormone deficiency in adults, infertility, treatment of burns, wound healing, dystrophy, bone knitting, osteoporosis, diffuse gastric bleeding, and pseudoarthrosis.

Furthermore, growth hormone has been proposed for increasing the rate of growth of domestic animals or for decreasing the proportion of fat in animals to be slaughtered for human consumption.

Pharmaceutical formulations of growth hormone tend to be unstable. Degradation products such as deamidated or sulfoxydated products and dimer or polymer forms are generated - especially in solutions of growth hormone.

The predominant degradation reactions of hGH are 1) deamidation by direct hydrolysis or via a cyclic succinimide intermediate to form various amounts of L-asp-hGH, L-iso-asp-hGH, D-asp-hGH, and D-iso-asp-hGH (ref 1-3), and 2) oxidation of the methionine residues in positions 14 and 125 (ref 4-9). The major degradation product of hGH in lyophilized state as well as in solution is deamidated hGH.

Deamidation especially takes place at the Asn in position 149 and to a minor extent in position 152.

hGH is also rather easily oxidized in positions 14 and 125.

The oxidation of hGH in solution forming sulfoxides is normally due to the oxygen dissolved in the formulation. The solubility of oxygen in distilled water is about 200 µM (9). As the concentration of hGH in a formulation comprising 4 IU/ml is 1.3 mg/ml corresponding to 60nM hGH, oxygen will, at normal storing conditions, be present in an excess of about 3000 times the stoichiometric amount for oxidation of

hGH. It is not feasible to try to solve the problem by degassing of buffers before tapping and packing the formulations.

At present, it is not believed that these deamidated forms and oxidized forms of hGH should have toxic or altered biological activity or receptor binding properties, but there is indication to the effect that the conformation stability of the sulfoxides is reduced as compared to native hGH.

For the development of a stable, dissolved formulation comprising hGH it is of importance to know the rate of deamidation and formation of sulfoxides as well as means to control the reactions.

The kinetics of degradation depend on temperature, pH and various additives or adjuvants in the hGH formulation.

Due to the instability, growth hormone is, at present, lyophilized and stored in the lyophilized form at 4°C until it is reconstituted for use in order to minimize the degradation.

The lyophilized pharmaceutical formulations comprising hGH are, at present, reconstituted by the patient and then stored as a solution during the use for a period of up to 14 days at 4°C, during which some degradation will take place.

Furthermore, the process of reconstitution of the lyophilized growth hormone tends to provide difficulties for the patient.

Thus, it is at present preferred to reconstitute the growth hormone as late as possible before use and to store and ship the formulation in a lyophilized state. The chain from the

manufacturer to the pharmacy is apt for handling the formulations at a controlled low temperature of e.g. 4°C which allows for a long shelf life of up to two years.

However, the extended use of pen systems for self-medication and the expanded field of use calls for a formulation which is stable for a sufficient long time with the end user under conditions where "sufficient" cooling is not always available.

Preferably, a formulation should be stable with the end user in a lyophilized state for about one month and additionally for one month in a reconstituted state in a pen device for the intended period of use of a cartridge.

Thus, there is a need for more stable formulations of growth hormone being stable in a lyophilized state at a relative high temperature for a period and additionally for a period of use at a relatively high temperature in solution. Such stabilization is of very great importance when moving the administration of the growth hormone from clinics to the homes of the individuals to be treated where optimal storage may not be available as indicated above.

Furthermore, the shift in pattern of administration of growth hormone to the use of pen devices calls for a stable dissolved formulation comprising growth hormone in order to facilitate the handling to be performed by the patient. A stable dissolved formulation comprising growth hormone may be produced ready to use in the form of cartridges fitting into the pen device used by the patient who may then avoid the reconstitution of the formulation and, hence, will not have to be in the possession of a lyophilized formulation, a suitable vehicle for reconstitution as well as the necessary skill and sterile equipment for sterile reconstitution of the formulation.

For safety reasons it will also be desirable to avoid the reconstitution of a lyophilized formulation just before the use of the formulation.

Furthermore, it would also be an advantage to avoid the lyophilization step in the production of growth hormone formulations. Lyophilization is a time consuming and costly process and is also often a "bottleneck" in the production due to the limited capacity of the freeze drier.

Thus, there is a need to reduce the rate of the degradation processes in order to allow for dissolved hGH formulations being stable during shelf life and during the period of use of up to one month.

Prior attempts to stabilize hGH has not fully succeeded in preventing the formation of dimer. The problems associated with dimer formation is e.g noted in Becker, G.W., Biotechnology and Applied Biochemistry 9, 478 (1987).

International Patent Publication No. WO 89/09614 and Australian patent application No. 30771/89 disclose a stable pharmaceutical formulation containing human growth hormone, glycine, and mannitol. Such a formulation shows improved stability during normal processing and storage in a lyophilized state as well as in the period of use after the reconstitution.

Published European patent application No. 303 746 discloses that animal growth hormone may be stabilized with various stabilizers to give decreased formation of insolubles and preservation of the soluble activity in aqueous environments, such stabilizers including certain polyols, amino acids, polymers of amino acids having a charged side group at physiological pH, and choline salts. Polyols are selected from the group consisting of non-reducing sugars,

sugar alcohols, sugar acids, pentaerythritol, lactose, water-soluble dextrans and Ficoll; amino acids are selected from the group consisting of glycine, sarcosine, lysine or salts thereof, serine, arginine or salts thereof, betaine, N,N,-dimethyl-glycine, aspartic acid or salts thereof, glutamic acid or salts thereof; a polymer of an amino acid having a charged side group at physiological pH may be selected from polylysine, polyaspartic acid, polyglutamic acid, polyarginine, polyhistidine, polyornithine and salts thereof; and choline derivatives are selected from the group consisting of choline chloride, choline dihydrogen citrate, choline bitartrate, choline bicarbonate, tricholine citrate, choline ascorbate, choline borate, choline gluconate, choline phosphate, di(choline)sulphate and dicholine mucate.

US patent specification No. 4,917,685 discloses a delivery device designed to be implanted comprising growth hormone stabilized using the same stabilizers as mentioned in EP 303746.

Published European patent application No. 374,120 discloses a stabilized formulation comprising hGH and a polyol having three hydroxy groups. Glycerol and tris(hydroxymethyl)aminomethane are mentioned. Furthermore, the presence of histidine hydrochloride as a buffer together with the polyol is disclosed.

International Patent Publication No. WO 92/00998 discloses that crystals may be formed crystallizing growth hormone with zinc.

International Patent Publication No. WO 93/12811 discloses stabilized formulations of growth hormone in the form of a lyophilized powder or an aqueous solution comprising asparagine.

International Patent Publication No. WO 93/12812 discloses stabilized formulations of growth hormone in the form of a lyophilized powder or an aqueous solution comprising histidine. In such formulations the deamidation is reduced by 25-30% as compared to a corresponding formulation of growth hormone comprising phosphate buffer. Furthermore, it is disclosed that crystals may be formed crystallizing growth hormone with zinc in the presence of histidine.

International Patent Publication No. WO 93/19776 discloses protein formulations comprising growth hormone comprising citrate as buffer substance being more stable than formulations comprising phosphate buffer. The formulations may also comprise amino acids such as glycine and alanine and/or mannitol or other sugar alcohols and/or glycerol and/or other carbohydrates and optionally a preservative such as benzyl alcohol.

International Patent Publication No. WO 94/03198 discloses a stable aqueous formulation containing human growth hormone, a buffer, a non-ionic surfactant, and, optionally, a neutral salt, mannitol, or, a preservative.

Published European patent application No. 177,478 discloses a prolonged release composition comprising zinc and somatotropin in a continuous phase with a biocompatible oil.

Published European patent application No. 216,485 discloses a sustained-release composition comprising zinc and somatotropin in a vehicle comprising a vegetable oil and an adjuvant, especially beeswax, aluminum monostearate, carnauba wax or paraffin.

Published European patent application No. 277,043 discloses recovering somatotropin from an aqueous solution by adding a salt of a transition metal and precipitation of an insoluble complex.

International Patent Publication No. WO 93/13792 discloses a delayed release implantable device comprising a somatotropin and zinc and optionally a basic side group containing amino acid solubilizing Zn-somatotropin, arginine, alanine, histidine and glutamic acid being mentioned as examples of amino acids.

International Patent Publication No. WO 92/17200 discloses stable growth hormone metal ion formulations in the form of dimers showing stability to denaturation. The formulations may comprise glycine and optionally additionally alanine, glutamine, asparagine, arginine or lysine, such amino acids being particularly advantageous when lyophilizing the formulation to create a sufficient mass to form a stable, dry caked formulation.

BRIEF DESCRIPTION OF THE INVENTION

It has now surprisingly been found that a formulation of human growth hormone with zinc and optionally lysine or calcium ions before preparation of the final formulation shows a very high stability against deamidation in aqueous solution and is furthermore readily dissolvable in aqueous solvents when lyophilized. The stability of the product allows for the storing and shipment thereof in a lyophilized state or in the form of a dissolved or re-dissolved formulation.

The pharmaceutical formulations of the invention may be formulated for administration in any suitable way, e.g. by parenteral or oral administration or administration to a mucosal membrane, e.g. nasal administration. The pharmaceutical formulation may be presented in the form of a dose comprised in a vial or cartridge or any other suitable container such as a prefilled syringe or a pen device.

Thus, the formulation of the invention may be in the form of a lyophilized powder to be reconstituted later using conventional vehicles such as distilled water or water for injection or in the form of a solution comprising growth hormone. Such vehicles may comprise conventional preservatives such as phenol, m-cresol and benzyl alcohol.

A preferred embodiment of the invention is in the form of a pharmaceutical formulation of human growth hormone pre-treated with zinc and optionally lysine or calcium ions and further comprising a carrier in the form of a buffered aqueous solution of growth hormone. Such formulation is in a ready-to-use form and may be stored and shipped as an aqueous solution without any considerable degradation.

A buffer to be used in a solution of pre-treated growth hormone may e.g. be histidine, citrate, tartrate or phosphate buffer. Preferably, the buffer is histidine buffer.

The pre-treatment solution is preferably adjusted to a value in the interval from about 2 to about 9, more preferred to pH from 6 to 8, especially to about 7-7.3.

For stability reasons the pH in the final formulation of pre-treated growth hormone is preferably adjusted to a value of about 2 to about 9, more preferred to a value of about 6 to about 8, especially to a value of about 6.0 to about 6.8.

In order to obtain the stabilizing effect of zinc and optionally lysine or calcium ions zinc is preferably added in an amount of up to 2mM, preferably from about 1 to about 4 moles Zn per mol of growth hormone, preferably about 1 to 2 moles Zn per mole growth hormone, most preferred about 1 mol Zn per mol growth hormone.

Zinc is preferably added in the form of a physiologically acceptable soluble salt such as the chloride.

Calcium ions when present are preferably added in the form of a physiologically acceptable soluble salt such as the chloride.

The pharmaceutical formulation of the invention may furthermore comprise salts or sugar alcohols for adjusting the tonicity and optionally an excipient in order to facilitate the processing thereof, e.g. lyophilization and the rapid and complete dissolution of a lyophilized formulation when reconstituting the formulation before use.

An excipient may be selected from disaccharides such as lactose, trehalose, and sucrose, sugar alcohols such as sorbitol or mannitol, polysaccharides such as the polymers commercialized as Dextran® products such as Dextran® 40, Dextran® 70 or Dextran® 75, and Ficoll® and polyvalent alcohols such as polyethylene glycol or polyvinyl alcohol or a combination of two or more of these.

In a further aspect the invention relates to a method of preparing a pharmaceutical formulation comprising a growth hormone pre-treated with zinc and optionally lysine or calcium ions wherein the growth hormone is dissolved in a solution comprising zinc and optionally lysine or calcium ions by dissolving zinc chloride in deionized water optionally containing lysine or calcium ions, letting the solution stand for a while, adding the growth hormone and optionally adjusting the pH to from about 2 to about 9.

The pH may be adjusted by adding an acid which has no adverse effect on the growth hormone, preferably a physiologically acceptable acid e.g. a mineral acid such as hydrochloric acid, sulphuric acid or nitric acid or an organic acid such as acetic acid.

In an embodiment of the method of the invention, is added optionally salts and an excipient, whereafter the solution is filled into a container and lyophilized.

Still another aspect of the invention relates to the use of zinc and optionally lysine or calcium ions for pre-treatment of growth hormone for the formulation of a stabilized formulation of growth hormone.

In yet another aspect the invention relates to a method for treating a disorder affectable by growth hormone comprising administering a formulation which comprises a growth hormone pre-treated with zinc and optionally lysine or calcium ions.

In the present context "growth hormone" may be growth hormone from any origin such as avian, bovine, equine, human, ovine, porcine, salmon, trout or tuna growth hormone, preferably bovine, human or porcine growth hormone, human growth hormone being most preferred. The growth hormone used in accordance with the invention may be native growth hormone isolated from a natural source, e.g. by extracting pituitary glands in a conventional manner, or a growth hormone produced by recombinant techniques, e.g as described in E.B. Jensen and S. Carlsen in Biotech and Bioeng. 36, 1-11 (1990). The "growth hormone" may also be a truncated form of growth hormone wherein one or more amino acid residues has (have) been deleted; an analogue thereof wherein one or more amino acid residues in the native molecule has (have) been substituted by another amino acid residue, preferably a natural amino acid residue, as long as the substitution does not have any adverse effect such as antigenicity or reduced action; or a derivative thereof, e.g having an N- or C-terminal extension such as Met-hGH. The preferred growth hormone is hGH.

The term "dose" of growth hormone refers to that amount that provides therapeutic effect in an administration regimen. The formulations hereof are prepared containing amounts of hGH at least about 0.1 mg/ml, preferably upwards of about 10 mg/ml, preferably from about 1 mg/ml to about 40 mg/ml, more preferably from about 1 mg/ml to about 25 mg/ml, e.g. from 1 mg/ml to about 5 mg/ml, calculated on the ready-to-use formulation. For use of these compositions in administration to human beings suffering from hypopituitary dwarfism, for example, these formulations contain from about 0.1 mg/ml to about 10 mg/ml, corresponding to the currently contemplated dosage regimen for the intended treatment. The concentration range is not critical to the invention and may be varied by the physician supervising the administration.

Lysine to be used in accordance with the present invention is preferably the naturally occurring alpha amino acid. The lysine may be l or d lysine or a mixture thereof.

In the present context "high stability" is obtained when the formulation is more stable than the conventional formulation comprising phosphate buffer and preferably as stable as a corresponding formulation comprising histidine as stabilizer in which the deamidation of hGH is reduced by approximately 20% as compared with phosphate buffer as disclosed in WO 93/12812.

The solvent used in the method of the invention may be water, alcohols such as ethyl, n-propyl or isopropyl, butyl alcohol or mixtures thereof. The solvent may comprise a preservative such as phenol, m-cresol or benzyl alcohol.

The term "pre-treated" is used in the present context in connection with growth hormone formulations to designate a growth hormone which is treated with zinc and optionally lysine or calcium ions before the addition of or to the further components for preparation of a growth hormone formulation.

DETAILED DESCRIPTION OF THE INVENTION

The invention is explained more in detail in the below Examples which illustrate the invention. They are not to be considered as limiting the scope of the invention being defined by the appended claims.

EXPERIMENTAL PART

EXAMPLE

Reduction of the deamidation.

The rate of deamidation after different pre-treatments was examined at 37°C for hGH formulations containing 4 mg/ml hGH, 0.18 mM $ZnCl_2$, 3 mM histidine, 1.5% benzyl alcohol, pH 6.8 as compared to histidine and phosphate buffer at pH 6.8.

The hGH formulations were prepared by dissolving 24 mg hGH in 2.5 ml of

a) 2 mM $CaCl2_2$ + 0.36 mM $ZnCl_2$, pH 7-7.3,
b) 2 mM lysine + 0.36 mM $ZnCl_2$, pH 7-7.3 or
c) 0.36 mM $ZnCl_2$, pH 7-7.3.

After storage 1 h at 4°C (to allow Zn^{++} to complex with hGH) the preparations a)-c) were reformulated using a PD10 desalting column (Pharmacia) into 3 ml 6 mM histidine, 0.36 mM $ZnCl_2$, 8 mg/ml hGH. Thereafter 3 ml 3% benzyl alcohol were added resulting in a final formulation of 4 mg/ml hGH, 3 mM

histidine, 0.18 mM $ZnCl_2$, 1.5% benzyl alcohol, (pH adjusted to 6.8 adding HCl/NaOH).

As reference formulations were used a) 4mg/ml hGH, 3 mM histidine, 0.18 mM $ZnCl_2$ 1.5% benzyl alcohol, pH 6.8; b) 4mg/ml hGH, 3 mM histidine, 1.5% benzyl alcohol, pH 6.8; and c) 4 mg/ml hGH, 3 mM Na_2HPO_4, 1.5% benzyl alcohol, pH 6.8. The reference formulations were not pre-treated with Zn^{++}.

The hGH formulations stated in the below table were stored at 37°C for 7 days, and analyzed for the content of deamidated hGH by IE-HPLC. The results appear from the below table

Formulation	pH_{start} /pH_{end}	Content of deamidated hGH (%)#	Content of deamidated forms as compared with histidine (%)
3 mM histidine, 0.18 mM $ZnCl_2$, 1.5% benzyl alcohol, pH 6.8. Pre-treated in accordance with procedure a)	6.78/6.84	10.0	84
3 mM histidine, 0.18 mM $ZnCl_2$, 1.5% benzyl alcohol, pH 6.8. Pre-treated in accordance with procedure b)	6.75/6.83	11.3	88
3 mM histidine, 0.18 mM $ZnCl_2$, 1.5% benzyl alcohol, pH 6.8. Pre-treated in accordance with procedure c)	6.79/6.87	11.5	89
3 mM histidine, 0.18 mM $ZnCl_2$, 1.5% benzyl alcohol, pH 6.8. No pre-treatment.	6.84/6.92	12.8	99
3 mM histidine, 1.5% benzyl alcohol, pH 6.8	6.84/6.92	12.9	100
3 mM Na_2HPO_4, 1.5% benzyl alcohol, pH 6.8	6.83/6.86	15.9	123

#Desamido content corrected by 1% per 0.1 pH unit deviation from 6.8.

From the above table it appears that the deamidation rate of hGH is reduced to 84% relative to the histidine (68% relative to the phosphate formulation) when the hGH solution is treated with Zn^{++} in the presence of Ca^{++} or lysine prior to resalting into the final histidine formulation.

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CLAIMS

1. A pharmaceutical formulation comprising a growth hormone pre-treated with zinc and optionally lysine or calcium ions.

2. A pharmaceutical formulation as claimed in claim 1 further comprising a carrier in the form of a buffered aqueous solution of growth hormone pre-treated with zinc and optionally lysine or calcium ions.

3. A pharmaceutical formulation as claimed in claim 1 or 2 wherein the pH is adjusted to a value in the interval from about 2 to about 9.

4. A pharmaceutical formulation as claimed in any of the preceding claims wherein the concentration of zinc is up to about 2 mM.

5. A pharmaceutical formulation as claimed in any of the preceding claims further comprising salts and/or saccharides and/or sugar alcohols.

6. A pharmaceutical formulation as claimed in any of claims 1-5 wherein the growth hormone is hGH.

7. A method of preparing a pharmaceutical formulation comprising a growth hormone pre-treated with zinc and optionally lysine or calcium ions wherein the growth hormone is dissolved in a solution comprising zinc and optionally lysine or calcium ions by dissolving zinc chloride in deionized water optionally containing lysine or calcium ions, letting the solution stand for a while, adding the growth hormone, optionally adding a preservative, and optionally adjusting the pH to from about 2 to about 9.

8. A method as claimed in claim 7, wherein is added optionally salts and/or sugar alcohols and an excipient, whereafter the solution is filled into a container and lyophilized.

9. Use of zinc and optionally lysine or calcium ions for pre-treatment of growth hormone for the formulation of a stabilized formulation of growth hormone.

10. A method for treating a disorder affectable by growth hormone deficiency, comprising administering the formulation of claim 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00293

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/27, A61K 33/30
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REG, CAPLUS, MEDLINE, WPIDS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9217200 A2 (GENENTECH, INC.), 15 October 1992 (15.10.92) --	1-9
A	US 4816568 A (EDWIN J. HAMILTON, JR. ET AL), 28 March 1989 (28.03.89) -- ---	1-9

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search	Date of mailing of the international search report
17 October 1996	21 -10- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK96/00293

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 10
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/10/96

International application No.
PCT/DK 96/00293

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A2- 9217200	15/10/92	AU-A- 1757092	02/11/92
US-A- 4816568	28/03/89	NONE	